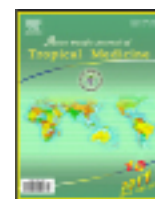


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In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant *Ocimum* species against *Plasmodium falciparum*

Samuel Jacob Inbaneson, Sundaram Ravikumar*, Palavesam Suganthi

Department of Oceanography and Coastal Area Studies, School of Marine Sciences, Alagappa University, Thondi Campus, Thondi – 623 409, Ramanathapuram District, Tamil Nadu, India

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ABSTRACT

Objective: To identify the possible antiplasmodial compounds from leaf, stem, root and flower extracts of *Ocimum canum* (*O. canum*), *Ocimum sanctum* (*O. sanctum*) and *Ocimum basilicum* (*O. basilicum*). **Methods:** The *O. canum*, *O. sanctum* and *O. basilicum* were collected from Ramanathapuram District, Tamil Nadu and the extraction was carried out in ethanol. The filter sterilized extracts (100, 50, 25, 12.5, 6.25 and 3.125 μ g/mL) of leaf, stem, root and flower extracts of *O. canum*, *O. sanctum* and *O. basilicum* were tested for antiplasmodial activity against *Plasmodium falciparum* (*P. falciparum*). The potential extracts were also tested for their phytochemical constituents. **Results:** The leaf extract of *O. sanctum* showed excellent antiplasmodial activity (IC₅₀ 35.58 μ g/mL) followed by leaf extract of *O. basilicum* (IC₅₀ 43.81 μ g/mL). The leaf extract of *O. canum*, root extracts of *O. sanctum* and *O. basilicum*, the stem and flower extracts of all the three tested *Ocimum* species showed IC₅₀ values between 50 and 100 μ g/mL. Statistical analysis reveals that, significant antiplasmodial activity ($P < 0.01$) was observed between the concentrations and time of exposure. The chemical injury to erythrocytes was also carried out and it shows that, there were no morphological changes in erythrocytes by the ethanolic extract of *O. canum*, *O. sanctum* and *O. basilicum*. The *in vitro* antiplasmodial activity might be due to the presence of alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins in the ethanolic extracts of tested plants. **Conclusions:** The ethanolic leaf extracts of *O. sanctum* possess lead compounds for the development of antiplasmodial drugs.

1. Introduction

Medicinal plants constitute an effective source of both traditional and modern medicine. Studies on plant secondary metabolites have been increasing over the last 50 years. Moreover, pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders worldwide[1]. The medicinal plant species *Ocimum canum* (*O. canum*), *Ocimum sanctum* (*O. sanctum*) (Holy Basil) and *Ocimum basilicum* (*O. basilicum*) belonged to Lamiaceae family are aromatic herb found throughout India. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *O. sanctum* have been recommended for the treatment of bronchitis, bronchial

asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite[2]. Scientifically, the *Ocimum* species have proved to possess various biological activities such as antibacterial[3,4], antifungal[5,6], antioxidant[7,8], antiviral[9] and anti-diabetic[10,11]. The emergence of strains of *Plasmodium falciparum* (*P. falciparum*), resistant to chloroquine and many other drugs in succession has stimulated efforts to identify new antimalarial agents. Previous discoveries and bioactive potential of *Ocimum* species insisted us to choose *O. canum*, *O. sanctum* and *O. basilicum* for the present study. In this connection, the present study was made an attempt to explore the antiplasmodial potential of *O. canum*, *O. sanctum* and *O. basilicum* against *P. falciparum*.

2. Material and methods

2.1. Plant material

Fresh samples of different plant parts (leaves, stem, root and flower) from *O. canum* (Lat. 9°21'41" N and Long. 78°49'45" E), *O. sanctum* (Lat. 9°21'45" N and Long. 78°49'41" E) and *O. basilicum* (Lat. 9°21'43" N and Long.

*Corresponding author: Dr. Sundaram Ravikumar, Reader, School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623 409, Ramanathapuram District, Tamil Nadu, India.

Tel: +914561-243470

Mobile: 0091- 9003306959

E-mail: ravibiotech201321@gmail.com

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78°49'43" E) were collected from Ramanathapuram District, Tamil Nadu, India and were botanically authenticated by Prof. K. Kathiresan, Faculty of Marine Sciences, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. The percentage of extraction and yields were described in Table 1. A sample voucher specimen was deposited in the herbarium facility (Sponsored by Indian Council of Medical Research, New Delhi) maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamil Nadu, India. All the collected samples were washed twice with distilled water to remove the adhering dusts and other associated animals.

2.2. Extract preparation

Shade dried samples were subjected for percolation by soaking in ethanol. After 21 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (>45 °C) and then freeze dried (–80 °C) to obtain solid residue. The percentage of extraction was calculated by using the following formula:

% of extraction = Weight of the extract (g)/ Weight of the plant material (g) × 100.

The extracts of *Ocimum* species were screened for the presence of phytochemical constituents by following the standard methods^[12]. The ethanolic extracts were dissolved in dimethyl sulphoxide (HiMedia Laboratories Private Limited, Mumbai, India) and filtered through Millipore sterile filters (mesh 0.20 µm, Sartorius Stedim Biotech GmbH, Germany). The filtrates were used for testing at different concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL^[12].

2.3. Parasite cultivation

The antiplasmodial activity of plant extracts was assessed against *P. falciparum* obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. *P. falciparum* are cultivated in human O Rh⁺ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India)^[13] supplemented with O Rh⁺ serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, India) and 40 µg/mL of gentamycin sulphate (HiMedia Laboratories Private Limited, Mumbai, India). Hematocrits were adjusted at 5% and parasite cultures were used when they exhibited 2% parasitaemia^[12].

2.4. In vitro antiplasmodial assay

Filter sterilized extracts (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) were incorporated in 96 well tissue culture plate containing 200 µL of *P. falciparum* culture with fresh red blood cells diluted to 2% hematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% hematocrit, positive control was maintained with parasitized blood cells culture treated with chloroquine and artemether^[13]. Parasitaemia was evaluated after 48 h by Giemsa stain and the average percentage suppression of parasitaemia was calculated by the following formula:

Average % suppression of parasitaemia = Average % parasitaemia in control – Average % parasitaemia in test/ Average % parasitaemia in control × 100.

2.5. Antiplasmodial activity calculation and analysis

The antiplasmodial activities of ethanolic extracts were

expressed by the inhibitory concentrations (IC₅₀) of the drug that induced 50% reduction in parasitaemia compared to the control (100% parasitaemia). The IC₅₀ values were calculated (concentration of extract in X axis and percentage of inhibition in Y axis) using Office XP (SDAS) software with linear regression equation^[13]. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al*^[14]. According to this norms, an extract is very active if IC₅₀ < 5 µg/mL, active 5 µg/mL < IC₅₀ < 50 µg/mL, weakly active 50 µg/mL < IC₅₀ < 100 µg/mL and inactive IC₅₀ > 100 µg/mL. Analysis of variances was performed by ANOVA procedures followed by a specific posthoc test to analyze the difference and statistical significances were achieved when P<0.01.

2.6. Chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might be attributed to the extract, 200 µL of erythrocytes were incubated with 100 µg/mL of the extract at a dose equal to the highest used in the antiplasmodial assay. The conditions of the experiment were maintained as in the case of antiplasmodial assay. After 48 h of incubation, thin blood smears were stained with Giemsa stain and observed for morphological changes under high–power light microscopy. The morphological findings were compared with those in erythrocytes that were uninfected and not exposed to extract^[13].

3. Results

The percentage yields of crude extracts ranged from 2.42 to 16.79 (Table 1). It reveals that, leaf of *O. sanctum* (16.79%) showed maximum yield followed by leaf of *O. basilicum* (16.22%). The leaf extract of *O. sanctum* (IC₅₀ 35.58 µg/mL) showed excellent antiplasmodial activity and followed by leaf extract of *O. basilicum* (43.81 µg/mL). Moreover, the root extract of *O. canum* showed IC₅₀ value of more than 100 µg/mL (Table 2).

The microscopic observation of uninfected erythrocytes incubated with the ethanolic extracts and uninfected erythrocytes from the blank column of the 96–well plate showed no morphological differences after 48 h of incubation. The preliminary phytochemical studies reveals that, the extracts from *Ocimum* species have variety of phytochemical constituents, namely alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins (Table 3).

Table 1

Percentage yield of ethanolic extracts from *Ocimum* species.

Botanical name	Plant part	Weight of plant part(g)	Yield of extract	
			(g)	%
<i>O. canum</i>	Leaf	102	15.62	15.31
	Stem	59	3.57	6.05
	Roots	58	1.68	2.90
	Flower	42	1.73	4.12
<i>O. sanctum</i>	Leaf	78	13.10	16.79
	Stem	49	2.85	5.82
	Roots	55	1.51	2.75
	Flower	40	1.64	4.10
<i>O. basilicum</i>	Leaf	82	13.30	16.22
	Stem	52	3.25	6.25
	Roots	60	1.45	2.42
	Flower	45	1.86	4.13

Table 2IC₅₀ value of *Ocimum* species extracts against *P. falciparum*.

Botanical name	Plant part	IC ₅₀ (μ g/mL)
<i>O. canum</i>	Leaf	53.50
	Stem	63.19
	Roots	>100.00
	Flower	82.08
<i>O. sanctum</i>	Leaf	35.58
	Stem	53.50
	Roots	87.40
	Flower	71.91
<i>O. basilicum</i>	Leaf	43.81
	Stem	63.67
	Roots	78.69
	Flower	76.75
Chloroquine	–	18.63
Artemether	–	5.55

Values are found significant between concentrations and time of exposure ($P < 0.01$).

Table 3Preliminary phytochemical constituents of leaf extracts of *Ocimum* species.

Phytochemical constituents	Botanical name		
	<i>O. canum</i>	<i>O. sanctum</i>	<i>O. basilicum</i>
Alkaloids	+	+	+
Glycosides	+	+	+
Coumarins	–	–	–
Flavanoids	+	+	+
Quinones	–	–	–
Phenols	+	+	+
Saponins	+	+	+
Triterpenoids	+	+	+
Proteins	+	+	+
Resins	+	+	+
Steroids	+	+	+
Tannins	+	+	+

– : Absent; + : Present.

4. Discussion

Malaria is a major health problem in many developing countries. The drugs resistant *P. falciparum* causes the most virulent form of malaria in humans and it is described as a public health disaster causing increased morbidity and mortality. The development and spread of drug resistant strains of the causative agent *P. falciparum* have limited the effectiveness of the currently used malarial drugs. In Kenya, chloroquine has been discontinued as the first line treatment for malaria due to overwhelming presence of resistant *P. falciparum* strains[15]. This creates the urgent need for new antimalarial drugs. Plants have always been considered to be a possible alternative and rich source of new drugs, and most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates[16]. *Ocimum* species provide a rich source of structurally diverse secondary metabolites. Several studies have demonstrated that, *Ocimum* species are an excellent source of components

such as alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins have exhibited different biological activities[17–19].

Studies on antiplasmodial activities with *Ocimum* species extracts are too limited; in this connection, the present study was investigated with different plant parts of *O. canum*, *O. sanctum* and *O. basilicum*. Among the tested extracts, *O. sanctum* showed IC₅₀ value (35.58 μ g/mL) at 1.9 fold concentration of positive control chloroquine. This might be due to the presence of glycosides[20], alkaloids[21], triterpenoids[22], flavonoids[23], phenols[12], saponins[23], proteins[24], resins[13], steroids[25] and tannins[12]. According to Rasoanaivo *et al*[14], 17%, 75% and 8% of extracts from selected *Ocimum* species were classified as active, weakly active and inactive respectively. The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *O. sanctum* possess moderate activity against the chloroquine-sensitive (3D7) strain of *P. falciparum*[26]. *O. basilicum* and *O. suave* extracts showed good *in vitro* anti-malarial activity against the chloroquine-resistant strain of *P. falciparum*[27]. The methanolic leaf extract of *O. tenuiflorum* (IC₅₀ 31 μ g/mL) and *O. gratissimum* (IC₅₀ 32 μ g/mL) showed antiplasmodial activity against *P. falciparum*[28]. The methanol, water and dichloromethane extract of *O. americanum* and *O. gratissimum* showed antiplasmodial activity against chloroquine resistant strain W2 of *P. falciparum*[29]. The dichloromethane, dichloromethane/methanol (1:1), methanol and purified water extract of *O. americanum* showed antiplasmodial activity against *P. falciparum*[30].

The mechanism of action might be due to the inhibition of *P. falciparum* merozoites invasion into the erythrocytes[31] and disruption of *P. falciparum* rosettes[32] by the carbohydrates; inhibition of *P. falciparum* fatty acid biosynthesis[33], inhibition of hemozoin biocrystallization by the alkaloids[34], inhibition of protein synthesis by triterpenoids[35], inhibition of β -haematin formation[36], decreased mitochondrial membrane potential, DNA fragmentation and cytoplasmic acidification by steroids[37]. It is concluded from the present study that, ethanolic extracts from *Ocimum* species were shown for the first time to display *in vitro* antiplasmodial activity and warranted further investigation as potential sources of antiplasmodial agents. Additional *in vitro* and *in vivo* work aimed at understanding the mechanisms of action of the active plant species and isolating and characterising the bioactive constituents is underway in our laboratories and will be reported on in due course.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

- [1] Johnson M, Babu A, Irudayaraj V. Antibacterial studies on *in vitro* derived calli of *Ocimum basilicum* L. *J Chem Pharm Res* 2011; **3**(1): 715–720.
- [2] Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian J Physiol Pharmacol* 2005; **49**(2): 125–131.
- [3] Parag S, Vijayashree N, Ranu B, Patil BR. Antibacterial activity of *Ocimum sanctum* Linn. and its application in water purification. *Res J Chem Environ* 2010; **14**(3): 46–50.
- [4] Obinna NC, Nwodo CS, Olayinka AO, Chinwe IO, Kehinde OO. Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *Afr J Food Sci* 2009; **3**(3): 77–81.
- [5] Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. *World J Med Sci* 2008; **3**(2): 81–88.
- [6] Singh S. *In vitro* antifungal activity of some essential oils against food spoilage fungi. *J Herb Med Toxicol* 2010; **4**(2): 107–111.
- [7] Ramesh B, Satakopan VN. *In vitro* antioxidant activities of *Ocimum* species: *Ocimum basilicum* and *Ocimum sanctum*. *J Cell Tiss Res* 2010; **10**(1): 2145–2150.
- [8] Hanif MA, Al-Maskari MY, Al-Maskari A, Al-Shukaili A, Al-Maskari AY, Al-Sabahi JN. Essential oil composition, antimicrobial and antioxidant activities of unexplored *Omani basil*. *J Med Plants Res* 2011; **5**(5): 751–757.
- [9] Arora R, Chawla R, Marwah R, Arora P, Sharma RK, Kaushik V, et al. Potential of complementary and alternative medicine in preventive management of novel H1N1 flu (Swine Flu) pandemic: thwarting potential disasters in the bud. *Evid Based Complement Alternat Med* 2011; DOI: 10.1155/2011/586506.
- [10] Chandra A, Mahdi AA, Singh RK, Mahdi F, Chander R. Effect of Indian herbal hypoglycaemic agents on antioxidant capacity and trace elements content in diabetic rats. *J Med Food* 2008; **11**: 506–512.
- [11] Reddy SS, Karuna R, Baskar R, Saralakumari D. Prevention of insulin resistance by ingesting aqueous extract of *Ocimum sanctum* to fructose-fed rats. *Horm Metab Res* 2008; **40**: 44–49.
- [12] Ravikumar S, Jacob Inbaneson S, Suganthi P, Gnanadesigan M. *In vitro* antiparasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive *Plasmodium falciparum*. *Parasitol Res* 2011; **108**: 873–878.
- [13] Ravikumar S, Jacob Inbaneson S, Suganthi P, Venkatesan M, Ramu A. Mangrove plants as a source of lead compounds for the development of new antiparasmodial drugs from South East coast of India. *Parasitol Res* 2011; **108**: 1405–1410.
- [14] Rasoanaivo P, Ratsimamanga Urverg S, Ramanitrasimbola D, Rafatro H, Rakoto Ratsimamanga A. Criblage d'extraits de plantes de Madagascar pour recherche d'activite antipaludique et d'effet potentialisateur de la chloroquine. *J Ethnopharmacol* 1992; **64**: 117–126.
- [15] Dianne J, Jeanne M, Margarette S, Oren S, Aggrey J, Piet A, et al. Treatment history and treatment dose are important determinants of sulfadoxine-pyrimethamine efficacy in children with uncomplicated malaria in Western Kenya. *J Infect Dis* 2003; **187**: 467–468.
- [16] Basco LK, Mitaku S, Skaltsounis AL, Ravelomanantsoa N, Tillequin F, Koch M, et al. *In vitro* activities of acridone alkaloids against *Plasmodium falciparum*. *Antimicrobi Agents Chemother* 1994; **5**: 1169–1171.
- [17] Bihari CG, Manaswini B, Kumar JP, Kumar TS. Pharmacognostical and phytochemical investigation of various *tulsi* plants available in south eastern odisha. *Int J Res Pharm Biomed Sci* 2011; **2**(2): 605–610.
- [18] Koche D, Shirsat R, Imran S, Bhadange DG. Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (MS) India. *Int J Pharm Sci* 2010; **1**(4): 253–256.
- [19] Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, et al. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *J Microbiol Antimicrob* 2011; **3**(1): 1–7.
- [20] Ene AC, Atawodi SE, Ameh DA, Kwanashie HO, Agomo PU. *In vivo* antiparasmodial effect of chloroform extracts of *Artemisia maciverae* Linn and *Artemisia maritima* Linn. *Afr J Biotechnol* 2009; **8**(23): 6612–6616.
- [21] Oliveira AB, Dolabela MF, Braga FC, Jacome RLRP, Varotti FP, Povoia MM. Plant derived antimalarial agents: new leads and efficient phythomedicines. *Ann Bra Aca Sci* 2009; **81**(4): 715–740.
- [22] Moon HI, Jung JC, Lee J. Antiparasmodial activity of triterpenoids isolated from whole plants of *Viola* genus from South Korea. *Parasitol Res* 2007; **100**: 641–644.
- [23] Ramazani A, Sardari S, Zakeri S, Vaziri B. *In vitro* antiparasmodial and phytochemical study of five *Artemisia* species from Iran and *in vivo* activity of two species. *Parasitol Res* 2010; **107**: 593–599.
- [24] Ravikumar S, Jacob Inbaneson S, Suganthi P, Gokulakrishnan R, Venkatesan M. *In vitro* antiparasmodial activity of ethanolic extracts of seaweed macroalgae against *Plasmodium falciparum*. *Parasitol Res* 2011; **108**: 1411–1416.
- [25] Ravikumar S, Ramanathan G, Jacob Inbaneson S, Ramu A. Antiparasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. *Parasitol Res* 2011; **108**: 107–113.
- [26] Bagavan A, Rahuman AA, Kamaraj C, Kaushik NK, Mohanakrishnan D, Sahal D. Antiparasmodial activity of botanical extracts against *Plasmodium falciparum*. *Parasitol Res* 2011; **108**: 1099–1109.
- [27] Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kiama SG. Antimalarial herbal remedies of Msambweni, Kenya. *J Ethnopharmacol* 2010; **128**(2): 424–432.
- [28] Kamaraj C, Kaushik NK, Mohanakrishnan D, Elango G, Bagavan A, Zahir AA, et al. Antiparasmodial potential of medicinal plant extracts from Malaiyur and Javadhu hills of South India. *Parasitol Res* 2011; DOI: 10.1007/s00436-011-2457-6.
- [29] Kaou AM, Mahiou-Leddett V, Hutter S, Ainouddine S, Hassani S, Yahaya I, et al. Antimalarial activity of crude extracts from nine African medicinal plants. *J Ethnopharmacol* 2008; **116**: 74–83.
- [30] Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, et al. *In vitro* antiparasmodial activity of medicinal plants native to or naturalised in South Africa. *J Ethnopharmacol* 2004; **92**: 177–191.
- [31] Adams Y, Smith SL, Schwartz-Albiez R, Andrews KT. Carrageenans inhibit the *in vitro* growth of *Plasmodium falciparum* and cytoadhesion to CD36. *Parasitol Res* 2005; **97**: 290–294.
- [32] Rowe A, Berendt AR, Marsh K, Newbold CI. *Plasmodium falciparum*: a family of sulphated glycoconjugates disrupts erythrocyte rosettes. *Exp Parasitol* 1994; **79**: 506–516.
- [33] Tasdemir D, Topaloglu B, Perozzo R, Brun R, O'Neill R, Carballeira NM, et al. Marine natural products from the Turkish sponge *Agelas oroides* that inhibit the enoyl reductases from *Plasmodium falciparum*, *Mycobacterium tuberculosis* and *Escherichia coli*. *Bioorg Med Chem* 2007; **15**: 6834–6845.
- [34] Dubar F, Egan TJ, Pradines B, Kuter D, Ncokazi KK, Forge D, et al. The antimalarial ferroquine: role of the metal and intramolecular hydrogen bond in activity and resistance. *ACS Chem Biol* 2011; **6**(3): 275–287.
- [35] Kirby GC, O'Neil MJ, Philipson JD, Warhurst DC. *In vivo* studies on the mode of action of quassinoids with activity against chloroquine resistant *Plasmodium falciparum*. *Biochem Pharmacol* 1989; **38**: 4367–4374.
- [36] Pabon A, Deharo E, Zuluaga L, Maya JD, Saez J, Blair S. *Plasmodium falciparum*: effect of *Solanum nudum* steroids on thiol contents and beta-hematin formation in parasitized erythrocytes. *Exp Parasitol* 2009; **122**: 273–279.
- [37] Lopez ML, Vommaro R, Zalis M, De Souza W, Blair S, Segura C. Induction of cell death on *Plasmodium falciparum* asexual blood stages by *Solanum nudum* steroids. *Parasitol Int* 2010; **59**: 217–225.